Supporting Information

McCandless *et al.* 10.1073/pnas.0800898105

SI Text

Human CNS Tissues. Immunohistological studies were performed on formalin-fixed, paraffin-embedded archival specimens from two serologically proven cases of fatal West Nile virus (WNV) encephalitis retrieved from pathology files at St. Louis University School of Medicine. Samples were obtained in accordance with an Institutional Review Board approved protocol for human research. Sections (5 μm) were deparaffinized and immunostained as described (1), using antibodies at the following dilutions: CXCL12 at 1:66, CD3 at 1:50, and CXCR4 at 1:50. Immunoreactive complexes were detected via anti-rabbit or mouse biotin-conjugated antibodies augmented by streptavidin-horseradish peroxide and visualized by 3,3′-diaminobenzidine DAKO.

1. Engle MJ, Diamond MS (2003) Antibody prophylaxis and therapy against West Nile virus infection in wild-type and immunodeficient mice. *J Virol* 77:12941–12949.

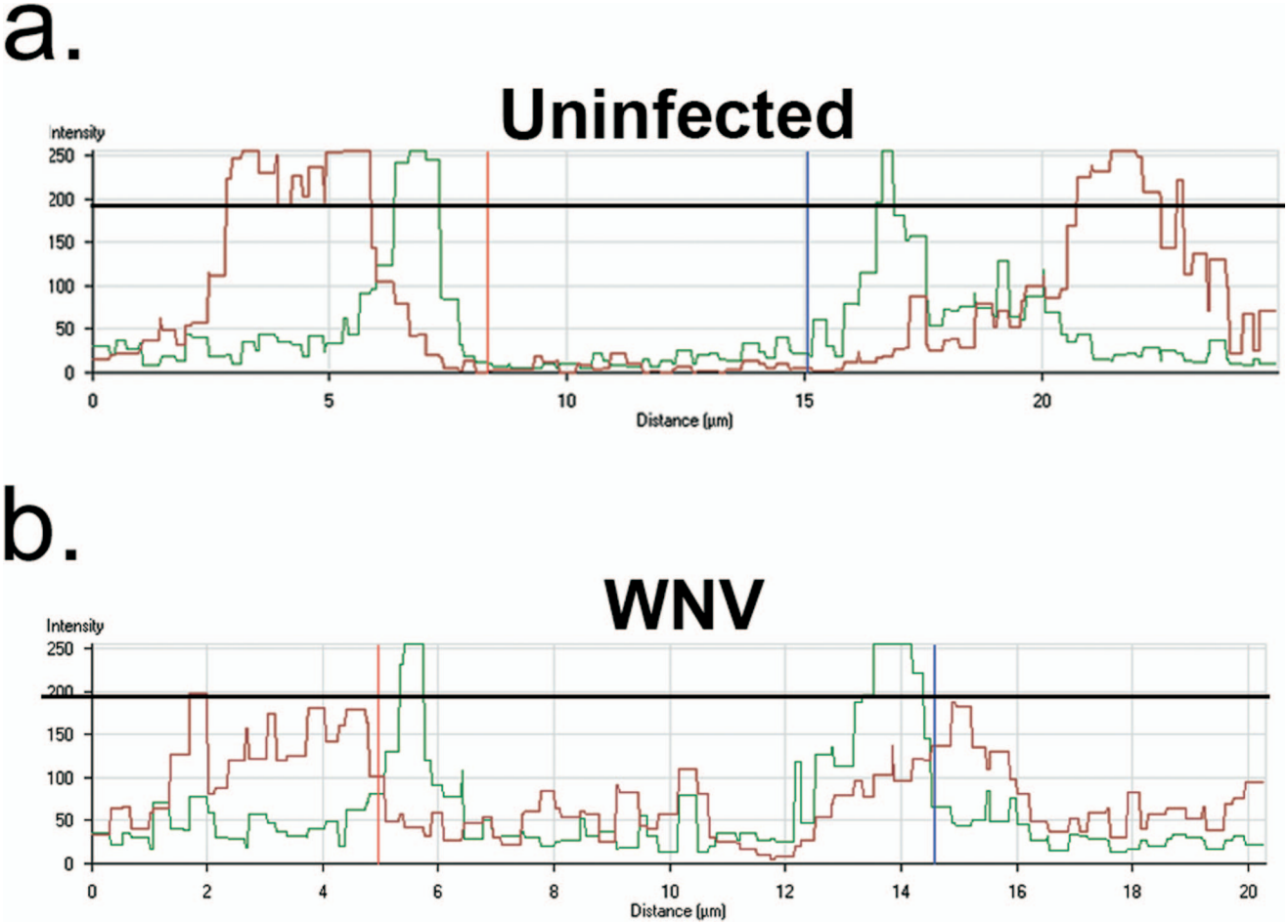

Fig. S1. Quantitative confocal analyses of CXCL12 at the BBB. Quantification of fluorescence intensity during confocal microscopy for CXCL12 (red stain and line) and CD31 (green stain and line) are shown. Double-headed arrows indicate the area transected with line plot depictions. The black line is set at the maximum CXCL12 intensity during WNV. Data are representative of 80–160 vessels, evaluated within uninfected and WNV-infected CNS tissues.

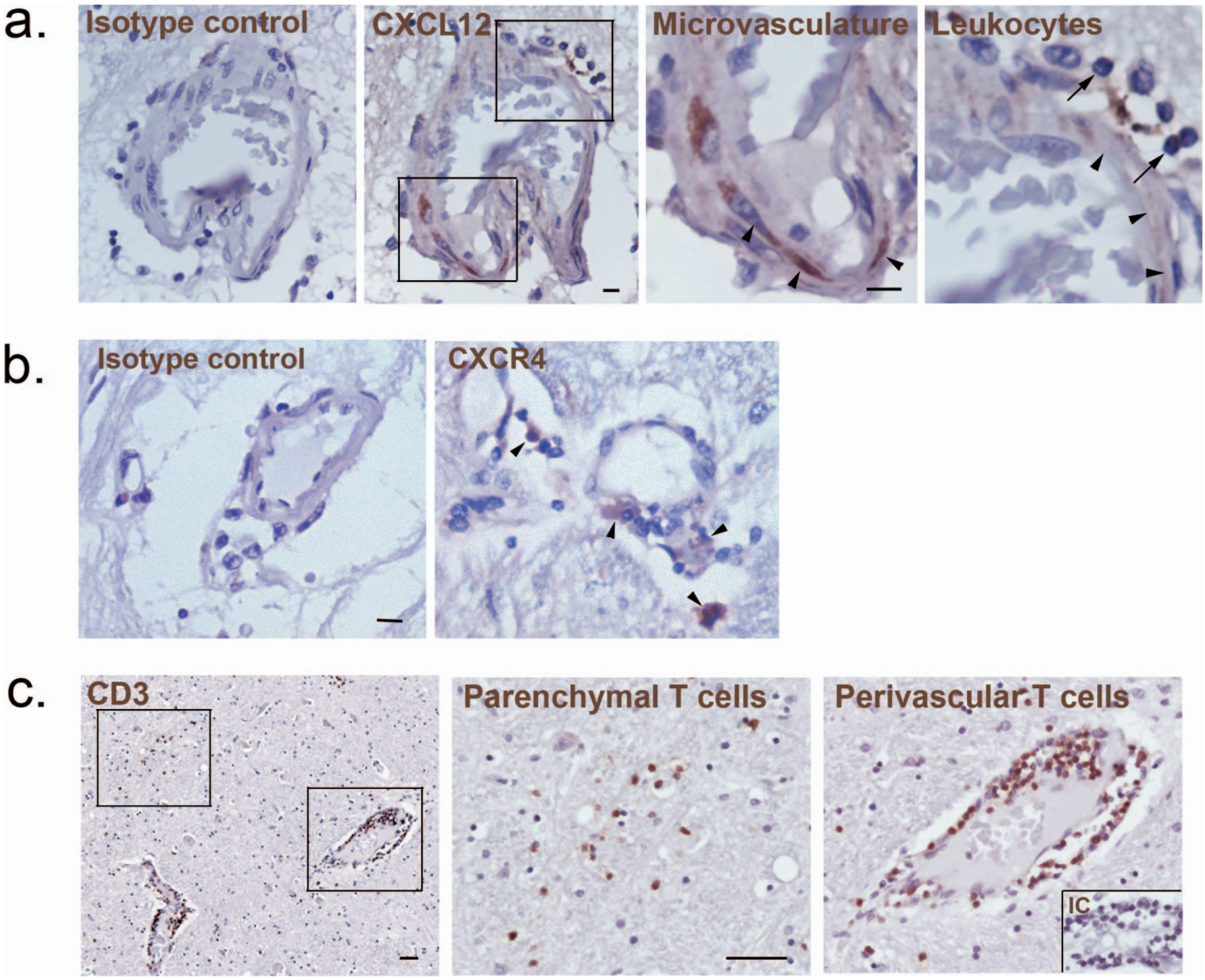

Fig. S2. CXCL12 and CXCR4 expression patterns in WNV human patients suggest the same perivascular retention of T cells as is seen in mice. Immunohistochemical analysis of brain sections collected from one of two patients with WNV encephalitis are shown. (*A*) Isotype control is shown in the first image while CXCL12 is shown in the second, third, and fourth images. The two left images depict low-power images of the vessel while the right two images are magnified, depicting CXCL12 expression along the microvasculature (arrowheads) and perivascular leukocytes (arrows). Representative images shown are counterstained with hemotoxylin (blue) and stained (brown). (*B*) Isotype control (*Left*) and CXCR4 (*Right*). Perivascular CXCR4+ leukocytes are depicted by arrowheads. Scale bar = 10 μm (*C*) *Center* and *Right* images depict high-power images of areas boxed in the low-power image (*Left*).CD3 or isotype control (inset).

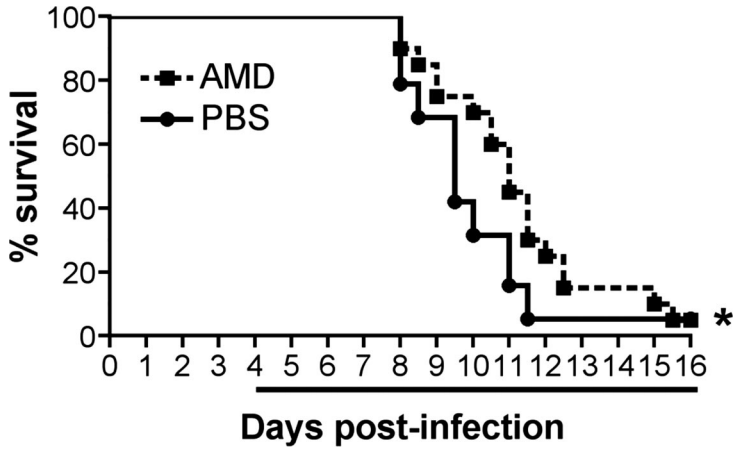

Fig. S3. Antagonism of CXCR4 and WNV infection. Mice were infected with 10 pfu of WNV and administered 100 μg AMD3100 (dashed line with squares, $n = 20$) or vehicle (PBS, solid line with circles, $n = 19$) beginning at day 4 via i.p. injection every 12 h until death. Statistical significance reflects the average survival time of animals in two independent experiments ($P < 0.05$). Overall survival rates between the two groups were not statistically significant ($P = 0.08$).

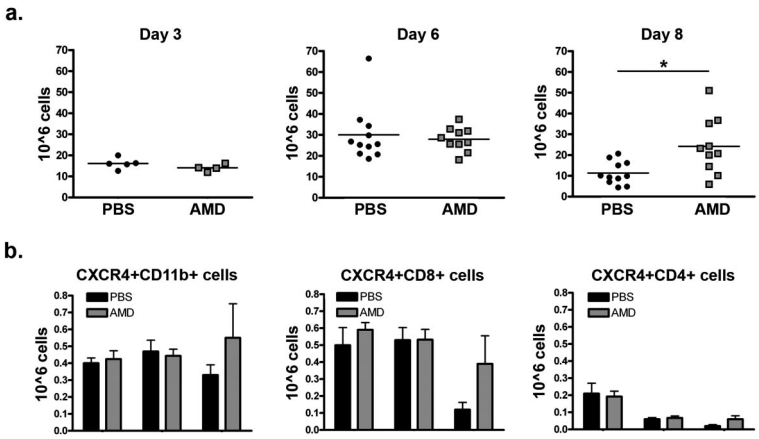

Fig. S4. CXCR4 antagonism does not affect cell trafficking in the spleen during WNV. (*A*) Total numbers of cells isolated from the spleens of PBS- (black circles) and AMD3100- (gray squares) treated mice on day 3 (*Left*), day 6 (*Center*), and day 8 (*Right*) after infection. (*B*) Total numbers of CXCR4+ splenocytes as determined by using flow cytometry, collected from PBS- (black bars) or AMD3100- (gray bars) treated mice between days 3, 6, and 8 after infection. (*Left*) Macrophage/microglia. (*Center*) CD8+ T cells. (*Right*) $CD4^+$ T cells. $n = 4–11$ animals per group. For all images, data shown consist of at least two independent experiments. *, $P < 0.05$.